

application as originally filed. No new matter is believed to have been introduced by the submission of the Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Docket No.:	<u>205645US20</u>
Serial No.:	<u>09/824,735</u>
Amendment Filed:	<u>08/17/01</u>

IN THE SPECIFICATION

Page 1, lines 8-9, please replace the paragraph with the following paragraph:

--This application claims benefit to U.S. provisional application No 60/194,649, filed on April 04, [2001] 2000, and incorporated herein by reference in its entirety.--

Page 6, lines 1-6, please replace the paragraph with the following paragraph:

--Fig. 2: SOS2 encodes a putative serine/threonine protein kinase. (A) Diagrammatic representation of SOS2 structure. (B) SOS2 cDNA sequence (SEQ ID NO:1) and the conceptual translation product (SEQ ID NO:2) of its longest ORF (GenBank accession number AF 237670). Underlined is a stop codon (TAA) at -6 to -4 that precedes the ATG in-frame. Numbers I-XI indicate kinase subdomains as defined by Hanks et al. (25), with invariant and nearly invariant amino acid residues highlighted in black and gray, respectively.--

Page 6, lines 7-13, please replace the paragraph with the following paragraph:

--Fig. 3: Amino acid alignments. (A) Alignment of putative kinase catalytic domain of SOS2 with *Saccharomyces cerevisiae* SNF1, SEQ ID NO:3, (23) and human AMPK kinases, SEQ ID NO:4 (24). Amino acid residues identical in at least two proteins are highlighted in black and conservative substitutions in gray. Mutations that abolish SOS2 autophosphorylation (see Fig. 4) are indicated; first * is K40N,m and second is * G197E, which corresponds to the *sos2-5 allele*. (B) Alignment of the C-terminal portion of SOS2

with the regulatory domains of *Schizosaccharomyces pombe* (yCHK1, SEQ ID NO:5) and human CHK1 (hCHK1, SEQ ID NO:6) kinases (27).--

Page 13, prenumbered lines 4-24, please replace the paragraph with the following paragraph:

--**Protein Expression.** To produce bacterially expressed recombinant proteins, the coding region of *SOS2*, *SOS2*(K40N), and *SOS2*(G197E) cDNAs were amplified by PCR with primers harboring restriction sites, cloned in frame into *Bam*HI-*Eco*RI of pGEX-2TK (Amersham Pharmacia), and transformed into *Escherichia coli* BL21 DE3 cells (Amersham Pharmacia). Mutations K40N and G197E in the *SOS2* protein were created by site-directed mutagenesis. For glutathione S-transferase (GST)-*SOS2*(K40N), primer pairs 5'-GCGGATCCATGACAAAGAAAATGAGAAGAGTGGGC (SEQ ID NO:7) and 5'-ATTGTACTCTTAGCCATAATGTTGATGGCT (SEQ ID NO:8) were used for the first PCR, and 5'-GCGAATTCTTAAGTTGGGATCAAAACGTGATTGTTCTG (SEQ ID NO:9) and 5'-GTGATAATGTAGCCATCAACATTATGGCTA (SEQ ID NO:10) were used for the second PCR. For the mutant protein GST-*SOS2* (G197E), primer pairs 5'-GCGGATCCATGACAAAGAAAATGAGAAGAGTGGGC (SEQ ID NO:11) and 5'-ATATAACGAAAAGAATAACCTCGCAAGACC (SEQ ID NO:12) were used for the first reaction and 5'-GCTGATATTGTTGCTTTCGAGGTTATTCTT (SEQ ID NO:13) and 5'-GCGAATTCTTAAGTTGGGATCAAAACGTGATTGTTCTG (SEQ ID NO:14) were used for the second reaction. The final amplification was done with 5'-GCGGATCCATGACAAAGAAAATGAGAAGAGTGGGC (SEQ ID NO:15) and 5'-GCGAATTCTTAAGTTGGGATCAAAACGTGATTGTTCTG (SEQ ID NO:16) on both templates. The final constructs were confirmed by sequencing *E. coli* cultures were induced

with 0.5 mM isopropyl β -D-thiogalactoside, and recombinant proteins were affinity-purified from bacterial lysates with glutathione-Sepharose beads (Amersham Pharmacia).--



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